Preparation and characterization of poly(L-lactic acid) foams

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A particulate-leaching method was developed to prepare highly porous biodegradable polymer membranes. It involves the casting of polymer/salt composite membranes followed by the dissolution of the salt. Poly(L-lactic acid) porous membranes of controlled porosity, surface/volume ratio, and crystallinity were prepared with sodium chloride, sodium tartrate or sodium citrate sieved particles. For salt weight fractions of 50 and 60 wt%, asymmetric membranes were formed, independent of salt particle size. When 70–90 wt% salt was used, the membranes were homogeneous with interconnected pores. The membrane properties were independent of the salt type and were only related to the salt weight fraction and particle size. The porosity increased with the salt weight fraction, and the median pore diameter increased as the salt particle size increased. The polymer/salt composite membranes could be quenched or annealed to yield amorphous or semicrystalline foams with desired crystallinity. All foams were 99.9 wt% salt free and had porosities as high as 0.93 and median pore diameters up to 150 μm.

(Keywords: poly(L-lactic acid); membranes; porosity)

INTRODUCTION

Cell transplantation was recently proposed as an alternative treatment to whole organ transplantation for failing or malfunctioning organs (e.g. liver, pancreas)[1]. For the creation of an autologous implant, donor tissue is harvested and dissociated into individual cells, and the cells are attached and cultured onto a proper substrate which is ultimately implanted at the desired site of the functioning tissue[2]. Because many isolated cell populations can be expanded *in vitro* using cell culture techniques, only a very small number of donor cells may be needed to prepare an implant[3]. Consequently, the living donor need not sacrifice an entire organ, thus expanding significantly the donor pool. However, isolated cells cannot form new tissues on their own[4]. Most primary organ cells are anchorage-dependent and require specific environments which very often include the presence of a supporting material to act as a template for growth[5]. The potential of cell transplantation was explored for the regeneration of several tissues including nerve[6], liver[7], pancreas[8], cartilage[9] and bone[10], with the aid of different biological and synthetic materials. From these studies, one infers that the success of any cell transplantation therapy relies on the development of suitable substrates for both *in vitro* and *in vivo* tissue culture.

Synthetic biodegradable polymers can provide temporary scaffolding for transplanted cells and by so doing allow the cells to secrete extracellular matrix (ECM), enabling a completely natural tissue replacement to occur[11]. Their major advantage over bodily ECM proteins, such as collagen and glycosaminoglycans, is the ease with which their properties can be tailored to the needs of a particular organ[12]. For example, their macromolecular structure can be designed so that they are completely degraded and eliminated as the need for an artificial support diminishes. An important class of biodegradable polymers includes poly(alpha ester)s like poly(lactic acid), poly(glycolic acid) and their copolymers, which are among the few synthetic polymers approved for human clinical use[13]. They are presently utilized as surgical suture materials[14] and in controlled release devices[15] among other medical and pharmaceutical applications. They are biocompatible and their degradation products are low molecular weight compounds, such as lactic acid and glycolic acid, which enter into normal metabolic pathways[16]. Furthermore, copolymers of poly(lactic-*co*-glycolic acid) offer the advantage of a large

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spectrum of degradation rates, from days to years, by simply varying the copolymer ratio of lactic acid to glycolic acid[17].

Poly(alpha ester)s have also been utilized in different forms to repair small diameter vascular grafts[18], skin[19], cartilage[20] and bone[21]. However, they were of low porosity which did not provide the necessary surface area and internal volume required to transplant large numbers of cells. The physical and chemical requirements of templates for tissue ingrowth are: (i) biocompatibility; (ii) promotion of cell adhesion; (iii) enhancement of cell growth; (iv) retention of differentiated cell function; (v) large surface area per volume; (vi) high porosity to provide adequate space for cell seeding, growth and ECM production; and (vii) a uniformly distributed and interconnected pore structure (this is important so that cells are easily distributed throughout the device and an organized network of tissue constituents can be formed).

We have developed a new processing method to prepare highly porous membranes of biodegradable polymers that satisfy most of the above requirements for cell transplantation. A solvent-casting and particulate-leaching technique with appropriate thermal treatment is presented here to prepare foams of synthetic biodegradable polymers with specific porosity, surface/volume ratio, pore size, and crystallinity for different applications. The technique is validated for poly(L-lactic acid) (PLLA) foams of desired structure and morphology.

EXPERIMENTAL

Materials

Poly(L-lactic acid) (PLLA) was supplied by Polysciences (Warrington, PA). Granular sodium chloride, sodium tartrate, and sodium citrate (Mallinckrodt, Paris, KY) were ground with an analytical mill (model A-10, Tekmar, Cincinnati, OH). The ground particles were sieved with USA Standard Testing Sieves (ASTM-11 Specification, Fisher Scientific, Pittsburgh, PA) with openings of 53 μm (No. 270), 106 μm (No. 140) and 150 μm (No. 100) placed on a sieve shaker (model 18480, CSC Scientific, Fairfax, VA). Chloroform and methylene chloride were furnished by Mallinckrodt. The mercury used in the porosimetry studies was triple distilled (Bethlehem Apparatus, Hellertown, PA).

Processing method

PLLA porous membranes were prepared by a solvent-casting particulate-leaching technique that consisted of the following steps.

(i) Sieved salt particles were added in a PLLA solution in chloroform (or methylene chloride) and the vortexed dispersion was cast in a 5 cm Petri dish. Sodium chloride, sodium tartrate and sodium citrate particles were utilized, which were insoluble in chloroform (and PLLA solutions). Five different polymer/salt compositions were used in our studies: 1.25 g PLLA and 1.25 g salt (50 wt% salt); 1 g PLLA and 1.5 g salt (60 wt% salt); 0.75 g PLLA and 1.75 g salt (70 wt% salt); 0.5 g PLLA and 2 g salt (80 wt% salt); and 0.25 g PLLA and 2.25 g salt (90 wt% salt). The total amount of PLLA and salt was 2.5 g. For the first three compositions (50, 60 and 70 wt% salt) 8 ml solvent were used, whereas for the last two (80 and 90 wt% salt) 4 ml were used. For each composition, sieved salt particles of three different sizes, d, were employed: $0 < d < 53$ μm, $53 < d < 106$ μm and $106 < d < 150$ μm.

(ii) The solvent was allowed to evaporate from the covered Petri dish over 48 h. Residual amounts of solvent were removed by vacuum drying at 100 μmHg and 25°C for 24 h.

(iii) The resulting PLLA/salt composite membranes were heated at a temperature above the PLLA melting temperature to ensure complete melting of the polymer crystallites formed during the previous step.

(iv) The melted PLLA membranes with dispersed salt particles were either annealed (cooled down to room temperature slowly at a controlled rate) or quenched (cooled down rapidly) to produce semicrystalline membranes with specific crystallinity and amorphous membranes, respectively.

(v) The PLLA/salt composite membranes were immersed in 250 ml distilled, deionized water on a shaker at 100 rev min^{-1} at 25°C for 48 h (the water was changed every 6 h) to leach out the salt.

(vi) The salt-free PLLA membranes were air-dried for 24 h, vacuum-dried at 100 μmHg for 48 h and stored in a desiccator under vacuum until use.

The membranes produced without any heat treatment were semicrystalline and had a reproducible degree of crystallinity (see Results and Discussion). Steps (iii) and (iv) of the heat treatment were optional and were only included to prepare membranes with desired crystallinity. Then, the Petri dish bottom was covered with an aluminium-backed overlay (Cole-Parmer, Chicago, IL) to prevent sticking of the membrane to the glass bottom upon heating.

For the preparation of amorphous PLLA membranes, after step (ii), the PLLA/salt composite membranes (in covered glass Petri dishes) were heated in a convection oven (Model OV-490A-3, Blue M, Blue Island, IL) at 195°C (which is approximately 15°C higher than the PLLA melting temperature) for 90 min. Then they were taken from the oven and were rapidly immersed in liquid nitrogen for 15 min. The quenched membranes were thawed at 25°C for 1 h before proceeding to step (v).

The exact value of the initial salt weight fraction was calculated from weight measurements of the composite membranes before they were immersed in water to leach out the salt (prior to step (v)) and after they had been vacuum dried (following step (vi)).

Mercury intrusion porosimetry

The pore size distribution of PLLA and PLLA/NaCl composite membranes was determined by a mercury intrusion porosimeter (model Poresizer 9220, Micromeritics, Norcross, GA). A solid penetrometer with 6 ml bulb volume (model 920-61707-00, Micromeritics) was used with porous membrane stripes of approximate dimension 1 cm × 2 cm and weight in the range of 0.01–0.06 g. (For composite membranes, the sample weight was between 0.06 and 0.1 g.) The values of void volume and pore area were calculated from measurements of the mercury intrusion volume and area at different pressures[22]. The filling pressure, P_{min}, of the penetrometer was 0.5 psi and the maximum pressure was 30 psi. At the pressure of 30 psi, the total intrusion volume had reached a plateau value. The effect of the polymer compressibility on the intrusion volume measurements

was negligible for pressures up to 30 psi[22] and was neglected. Thus, the reported values of cumulative void volume and pore area refer to pores with diameters smaller than that given by the Washburn equation[22]:

$$d_p = \frac{-4\gamma \cos\theta}{P_{min}} \quad (1)$$

where γ is the surface tension of mercury (485 dyn cm^{-1} at 25°C) and θ is the advance contact angle between the mercury and the pore wall. For PLLA, the value of θ was measured[23] at 25°C as 160°. The diameter, d_p, from equation (1) corresponds to an equivalent cylindrical pore and provides an approximation of the interstitial distance. Here, it is calculated as 529 μm.

The porosity, ε, was calculated from the total intrusion volume (per unit mass), V_i, and the skeletal density, ρ, as:

$$\varepsilon = \frac{V_i}{V_i + 1/\rho} \quad (2)$$

For PLLA porous membranes, the skeletal density was the polymer density, ρ_p, which is given by:

$$\rho_p = \frac{1}{(1-X_c)/\rho_a + X_c/\rho_c} \quad (3)$$

where X_c is the polymer degree of crystallinity measured by differential scanning calorimetry (d.s.c.), and ρ_a and ρ_c are the polymer densities of the amorphous and crystalline regions, respectively. For PLLA, $\rho_a = 1.248$ g ml^{-1} and $\rho_c = 1.290$ g ml^{-1} (ref. 24). For the special case of composite membranes, the skeletal density is given by:

$$\rho = \frac{1}{(1-w_s)/\rho_p + w_s/\rho_s} \quad (4)$$

where ρ_s is the salt crystal density and ρ_p is the polymer density calculated by equation (3). For sodium chloride, $\rho_s = 2.165$ g ml^{-1}. The parameter w_s is the salt weight fraction measured by thermogravimetric analysis (t.g.a.).

The surface/volume ratio, α, was calculated from the total intrusion area (per unit mass), A_i, as

$$\alpha = A_i \rho (1-\varepsilon) \quad (5)$$

Scanning electron microscopy (SEM)

The samples were coated with gold using a sputter coater (model Desk II, Denton Vacuum, Cherry Hill, NJ). The gas pressure was set at 50 mtorr and the current was 40 mA for a coating time of 75 s. A Hitachi (model S-530) scanning electron microscope was used in our studies and was operated at a 15 kV voltage.

Differential scanning calorimetry

A thermal analysis system (Perkin–Elmer, Series 7, Newton Centre, MA) was utilized to determine the thermal properties of PLLA/salt composite and PLLA porous membranes by measuring their melting and crystallization temperatures, and corresponding enthalpy changes. The sample weight for porous membranes was in the range of 2–12 mg, whereas for composite membranes approximately 20 mg of sample was tested. A heating (or cooling) rate of 10°C min^{-1} was applied in all studies. The degree of crystallinity, X_c, of a sample that exhibited no cold crystallization was calculated as:

$$X_c = \Delta H_m / \Delta H_m^\circ \quad (6)$$

where ΔH_m designates the measured enthalpy of melting and ΔH_m° the enthalpy of melting for 100% crystalline polymer. For PLLA, $\Delta H_m^\circ = 203.4$ J g^{-1} (ref. 25). Representative d.s.c. thermograms are shown in *Figure 1A, B*. The peak temperature of a melting endotherm is the melting temperature, T_m.

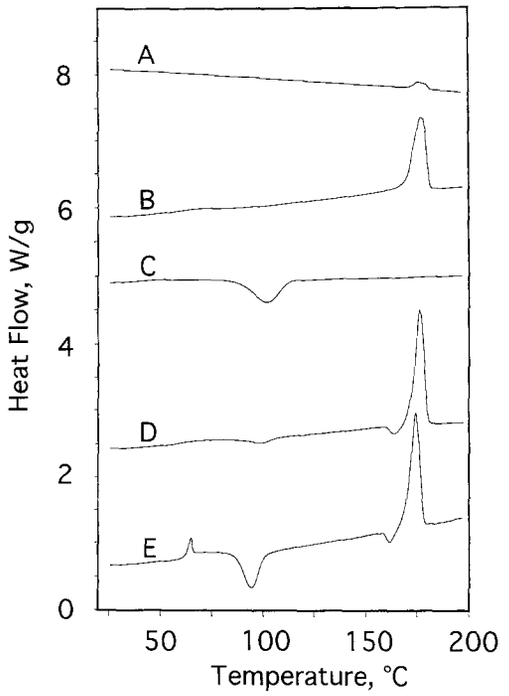

Figure 1 D.s.c. thermogram of a PLLA/NaCl composite (A). The thermograms B–D correspond to a semicrystalline PLLA foam heated from 20 to 200°C (B), kept at 200°C for 20 min, cooled to 20°C (C), kept at 20°C for 10 min, and heated to 200°C (D). Thermogram E is for an amorphous PLLA foam. In all cases, the heating or cooling rate was 10°C min^{-1}. For all samples, 90 wt% NaCl particles in the size range from 0 to 53 μm were utilized

For samples that were partially crystallized while tested at temperatures below T_m (a phenomenon named as cold crystallization), the intrinsic degree of crystallinity was corrected as follows:

$$X_c = \Delta H_m / \Delta H_m^\circ - \Delta H_c / \Delta H_c^\circ \quad (7)$$

The parameter ΔH_c stands for the measured enthalpy of crystallization and ΔH_c° for the enthalpy of crystallization required for 100% crystalline polymer. Both parameters refer to the same crystallization temperature, T_c, which is selected as the peak temperature of the crystallization exotherm.

A typical d.s.c. test involved the heating of a sample from 20 to 200°C to measure its melting temperature and enthalpy of melting. For the PLLA foams prepared without any heat treatment, the same sample was kept at 200°C for 20 min, cooled to 20°C, kept at 20°C for 10 min, and heated again to 200°C (second heating). An exotherm was recorded at 100.2 ± 1.7°C when the sample was cooled (*Figure 1C*) and at 103.9 ± 2.5°C when heated for the second time (*Figure 1D*), and the formed crystallites melted at 177.1 ± 1.0°C. The enthalpy of crystallization, ΔH_c°, was computed by equation (8) as 148.5 ± 6.1 J g^{-1}.

$$\Delta H_c^\circ / \Delta H_m^\circ = Q_{ex}/Q_{en} \quad (8)$$

Here, Q_{ex} is the total area under both exotherms and Q_{en} is the area under the second endotherm (second heating). (All values are averages ± standard deviation (s.d.) of 15 samples.)

Thermogravimetric analysis

The relative amounts of PLLA and salt of composite membranes were measured by t.g.a. (Perkin–Elmer, Series 7). In a typical experiment, 40 mg of material was heated from 150 to 550°C at a constant rate of 10°C min^{-1}, and the normalized sample weight (expressed as fraction of the initial weight at 150°C) was recorded as a function of temperature. For raw PLLA, the onset degradation temperature was 274.4 ± 4.8°C (average $\pm$ s.d. of three samples) and the peak degradation temperature was 315.2 ± 3.0°C. At 550°C, the normalized PLLA weight had reached a low plateau value of 0.0027 ± 0.0012, or approximately zero. The salt weight fraction, w_s, of a PLLA/NaCl composite membrane was equal to the normalized weight at 550°C.

Neutron activation analysis

Neutron activation analysis (n.a.a.), which is one of the most sensitive analytical techniques for identification and measurement of trace elements[26], was used to determine residual amounts of sodium in PLLA porous membranes. For this technique, a sample material is irradiated with subatomic particles resulting in the conversion of some of its nuclei to radioactive isotopes. (For example, ^{23}Na yields ^{24}Na.) The produced isotopes decay at a characteristic rate emitting γ-rays of characteristic energies. Membrane samples of approximately 40 mg were bombarded with thermal neutrons in a nuclear reactor (MITR-II) at the MIT Nuclear Reactor Laboratory. Standard reference materials were analysed along with the samples to calibrate the reactor and assess measurement accuracy. The sodium mass in a membrane sample[26] was proportional to the activity of γ-rays of energy 1368.5 keV. The minimum detection limit for the sodium weight fraction was 10^{-5}.

Gel permeation chromatography

The polymer molecular weight distribution was determined by gel permeation chromatography (g.p.c.) (Perkin–Elmer, Series 10) equipped with a refractive index detector (Perkin–Elmer, LC-25). The samples were dissolved in chloroform and eluted through a Phenogel guard column (model 22824G, 50×7.8 mm, particles 5 μm, mixed bed, Phenomenex, Torrance, CA) and a Phenogel column (model GP/4446, 300×7.8 mm, particles 5 μm, mixed bed, Phenomenex) at a flow rate of 1 ml min^{-1}. Polystyrene standards (Polysciences) were used to get a primary calibration curve. The values of the Mark–Houwink constants for PLLA, which were utilized to obtain the calibration curve and calculate the absolute polymer molecular weights[27], were $K = 5.45 \times 10^{-3}$ ml g^{-1} and $\alpha = 0.73$.

RESULTS AND DISCUSSION

Poly(L-lactic acid) (PLLA) porous membranes were prepared with sodium chloride, sodium tartrate and sodium citrate particles without any heat treatment to determine the effect of the initial salt weight fraction and particle size on the porosity, median pore diameter and surface/volume ratio. (The bulk volume of a membrane, i.e. that of pores plus solid, was used in the definition of the surface/volume ratio.) The membrane porosity increased monotonically with the initial salt weight fraction, and was independent of the salt particle size (*Figure 2*). For sodium chloride, duplicate samples were tested for each composition and the measured porosities, median pore diameters and surface/volume ratios were very reproducible (*Figure 2a*). Single measurements were performed for the remaining experiments. By varying the initial sodium chloride weight fraction from 0.5 to 0.9 (*Figure 2a*), the porosity of the PLLA membranes increased from 0.49 to 0.93 when salt particles of size 0–53 μm were used, from 0.50 to 0.92 for salt particles of size 53–106 μm, and from 0.48 to 0.91 for salt particles of size 106–150 μm. A similar dependence was also observed for PLLA membranes prepared with sodium tartrate (*Figure 2b*) or sodium citrate particles (*Figure 2c*).

The membrane porosity, ε, was calculated by equation (2) from measurements of the mercury intrusion volume. The salt particle size affected the pore size distribution. The median pore diameter of the porous membranes increased as the salt particle size increased. Nevertheless, it did not change with the initial salt weight fraction. For PLLA foams prepared with sodium tartrate particles of sizes 0–53, 53–106 and 106–150 μm (*Figure 2b*), the median pore diameter was 29 ± 9, 66 ± 7 and 116 ± 18, respectively (averages $\pm$ s.d. for foams prepared with 50, 60, 70, 80 and 90 wt% sodium tartrate).

The surface/volume ratio was calculated by equation (5), and was a function of both initial salt weight fraction and particle size. It increased with the salt weight fraction (thus, it increased with membrane porosity), and was much larger for smaller salt particles (or pore diameters). (For cylindrical pores, the surface/volume ratio scales to the inverse of pore diameter.) The PLLA foams prepared with 90 wt% sodium citrate (*Figure 2c*) had a surface/volume ratio of 0.119 μm^{-1} when particles of size range 0–53 μm were used, 0.081 μm^{-1} for particles of 53–106 μm, and 0.064 μm^{-1} for particles of 106–150 μm.

The salt type had no effect on the porosity, median pore diameter and surface/volume ratio of the porous membranes. The foam properties were independent of the salt used, and were only a function of the relative amount of salt and salt particle size.

Membranes prepared with salt weight fractions of 70 wt% and higher had a uniform pore morphology and the pores were evenly distributed, as evidenced by SEM photomicrographs of cross sections (*Figure 3*). The pores were interconnected, thus, yielding an open-cell polymer foam. It is apparent that the larger the initial salt weight fraction the larger the foam porosity. Also, by utilizing sieved salt particles of different sizes we prepared membranes of the same porosity but with different pore diameters (the porosity of the membranes shown in *Figures 3c* and *d* are 0.91 and 0.93, respectively). However, when 50 or 60 wt% salt was used, asymmetric membranes were formed with a dense impermable skin at the surface (see *Figure 4*). Here, the amount of polymer was much larger than that required to fill the crevices between the salt particles (which precipitated at the bottom of the Petri dish upon casting of the particle suspension).

The polymer/salt composite membranes were also porous. SEM photomicrographs of PLLA/NaCl membranes prepared with 80 wt% NaCl particles of different sizes reveal the existence of pores between salt particles (see *Figure 5*). Polymer fibrils and/or flakes bridge the surfaces of adjacent salt particles. The porosity of PLLA/NaCl composite membranes increased with the salt weight fraction, and was larger for smaller particles (*Figure 6*).

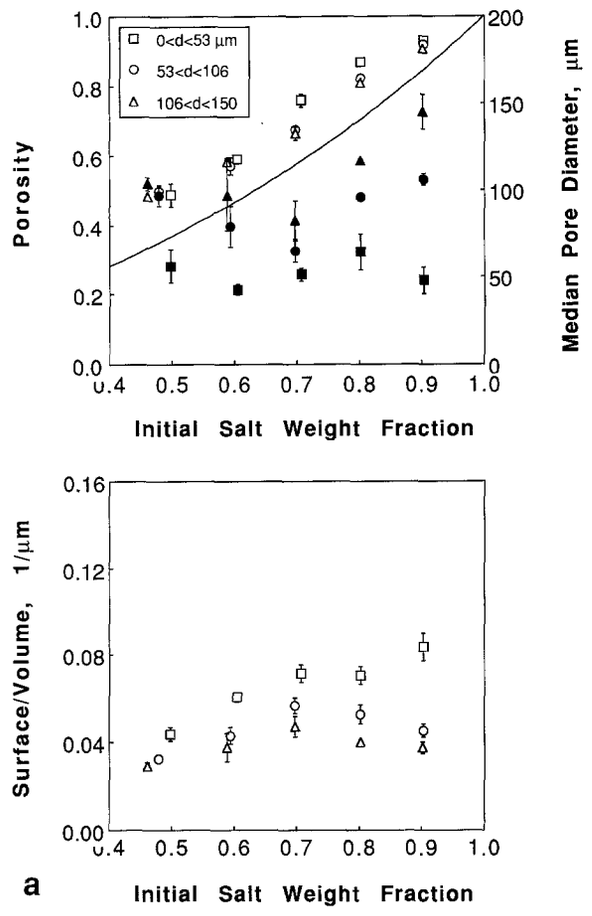

Figure 2 Porosity (top, open symbols), medium pore diameter (top, solid symbols), and surface/volume ratio (bottom) of semicrystalline PLLA foams prepared with sodium chloride (a), sodium tartrate (b), and sodium citrate particles (c) as a function of the initial salt weight fraction and salt particle size. □, $0 < d < 53\ \mu m$; ○, $53 < d < 106\ \mu m$; △, $106 < d < 150\ \mu m$. Theoretical predictions of the apparent porosity as a function of the initial salt weight fraction for sodium chloride (solid line) were calculated by equation (9). The error bars correspond to average ± the range of two measurements

The formation of porous composite membranes explains why (i) the foam porosity did not reach a plateau value (equal to one minus the porosity of a bed packed with salt particles) for large salt weight fractions, and (ii) theoretical predictions of an apparent porosity,

$$\varepsilon_{app} = \frac{w_s/\rho_s}{(1-w_s)/\rho_p + w_s/\rho_s} \tag{9}$$

assumed equal to the salt volume fraction of the composite membranes, were smaller than the corresponding experimental data (*Figure 2a*). Therefore, pores were not only created by the dissolution of salt particles but also during solvent evaporation and polymer solidification.

We compared the salt weight fraction of PLLA/NaCl composite membranes measured by t.g.a. with that calculated from the weights of the composite membranes and the produced porous membranes to evaluate the polymer connectivity of composite membranes (*Figure 7*). The two values were identical for salt weight fractions in the range of 50 to 90 wt% and particle sizes of 0–53,

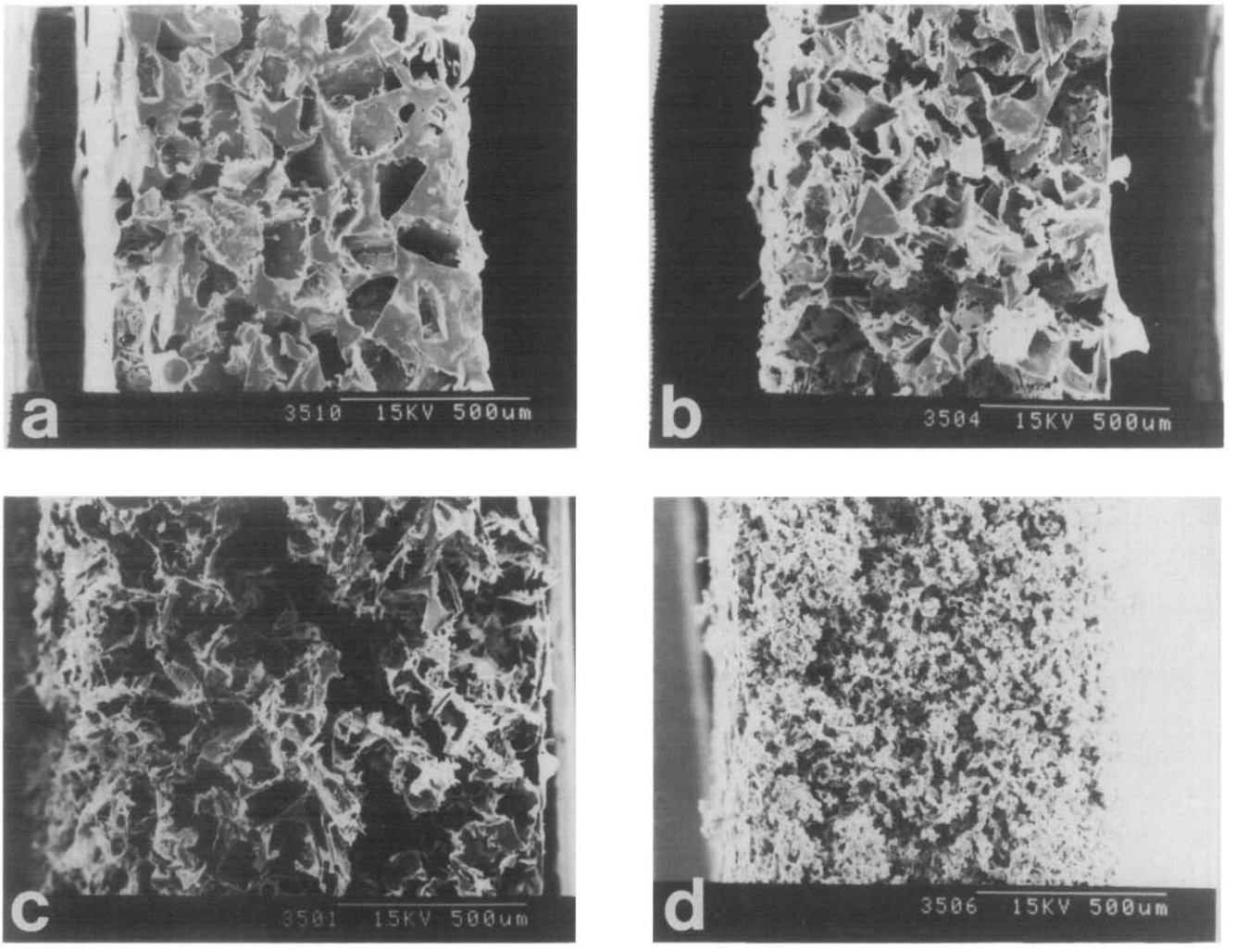

Figure 3 SEM photomicrographs of cross sections of semicrystalline PLLA foams prepared with 70 wt% (a), 80 wt% (b), and 90 wt% (c, d) NaCl particles in the size range from 106 to 150 μm (a, b, c) and from 0 to 53 μm (d)

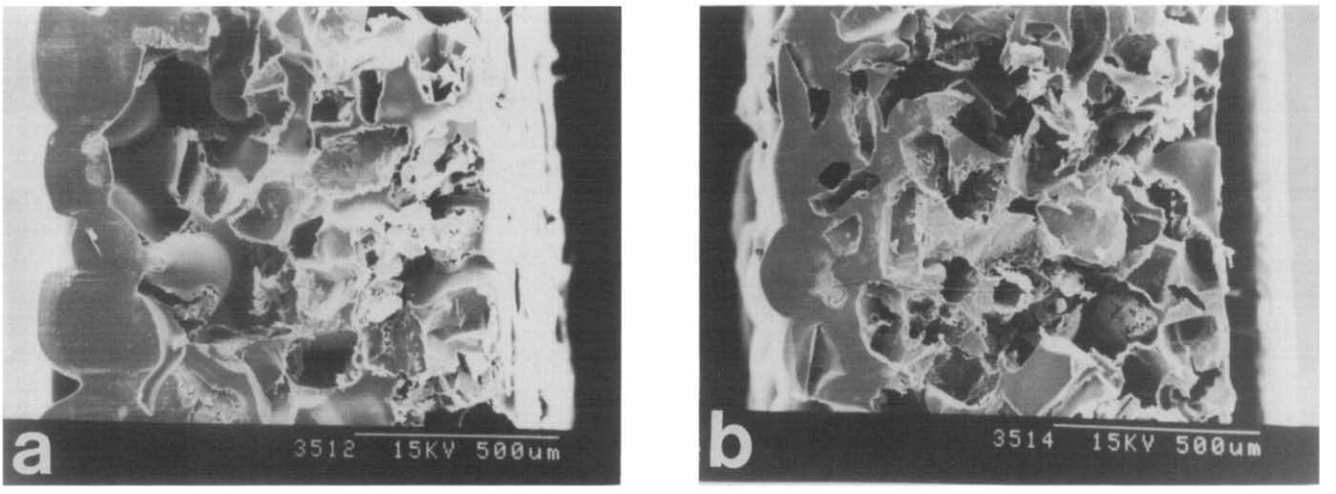

Figure 4 SEM photomicrographs of cross sections of semicrystalline PLLA asymmetric membranes prepared with 50 wt% (a) and 60 wt% (b) NaCl particles in the size range from 106 to 150 μm. The dense skin (left side) corresponds to the top surface of the membrane, which was exposed to air

53–106 and 106–150 μm, thus, confirming that no polymer was detached from the composite membrane during the salt-leaching step.

For salt weight fractions of 50, 60 and 70 wt%, 8 ml of chloroform was utilized whereas for 80 and 90 wt% salt 4 ml was used. The rationale for using 8 ml was to make the polymer solution less viscous and facilitate the casting procedure. Nevertheless, when 4 ml of chloroform was used for the weight fractions of 50, 60 and 70 wt%, the produced composite and porous membranes (data

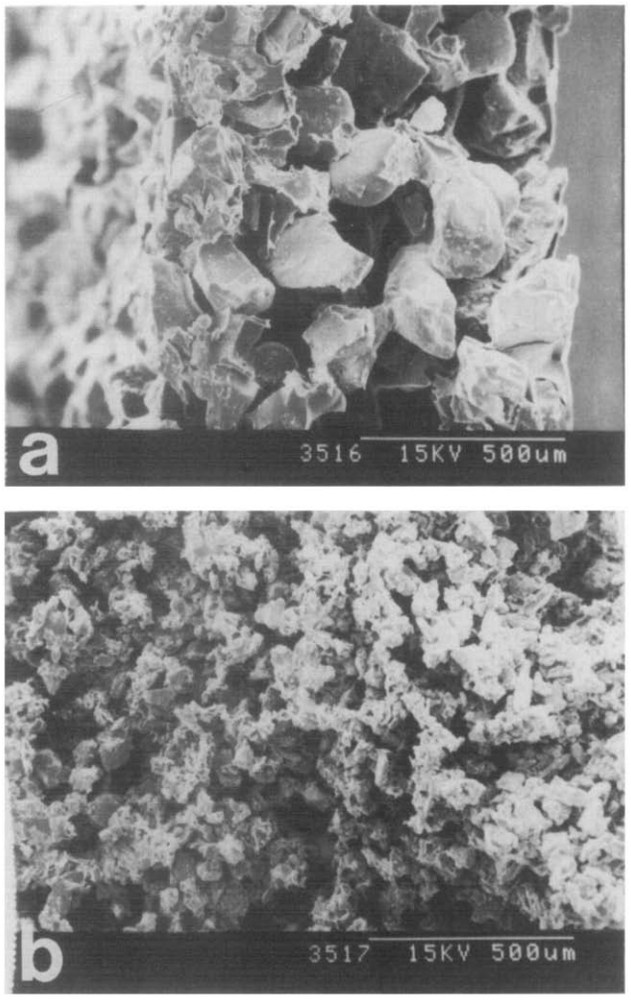

Figure 5 SEM photomicrographs of cross sections of PLLA/NaCl composites (before the salt-leaching step) prepared with 80 wt% NaCl particles in the size range from 106 to 150 μm (a) and from 0 to 53 μm (b)

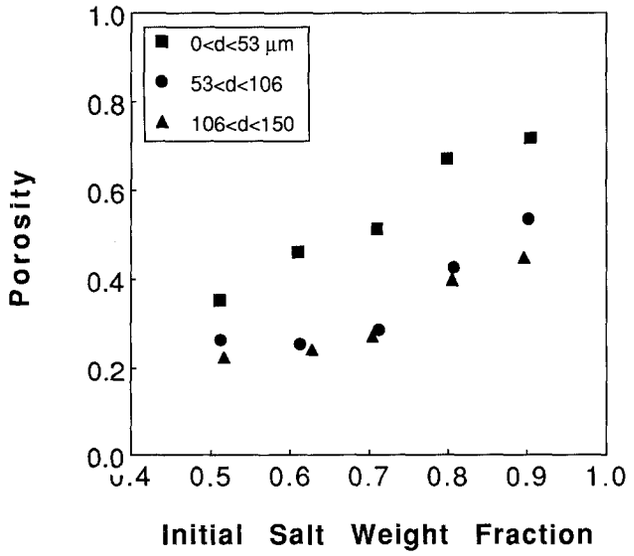

Figure 6 Porosity of PLLA/NaCl composites prepared with sodium chloride particles as a function of the initial salt weight fraction and salt particle size

not shown) had approximately the same porosities as those prepared with 8 ml of chloroform.

In all previous studies, the solvent for PLLA was chloroform. When methylene chloride was used, which is much more volatile than chloroform, again the composite and porous membranes had porosities similar to those prepared with chloroform (compare *Figures 2a* and *8*). Chloroform was used as a standard solvent because owing to its slow evaporation it could improve the chances of forming homogeneous membranes[28].

The skeletal density entered in the calculations of the porosity by equation (2) was derived from equation (3) or (4) as a function of the PLLA degree of crystallinity. All membranes prepared without any heat treatment exhibited no cold crystallization, and equation (6) was used to determine the value of X_c. The melting temperature(s), enthalpy of melting and degree of crystallinity of four representative PLLA/NaCl composite and PLLA porous membranes are presented in *Table 1*. The crystallites formed with solvent evaporation melted in the vicinity of 179.5 ± 1.5°C (average melting temperature $\pm$ s.d. of 15 samples). The crystallinity of the PLLA foams was the same as that of PLLA/NaCl composite membranes and was therefore not altered during the dissolution of the NaCl particles by water. The PLLA crystallinity was also not affected by the different means of varying the porosity or the surface/volume ratio as evidenced by exactly the same linear relationship between the heat of melting and

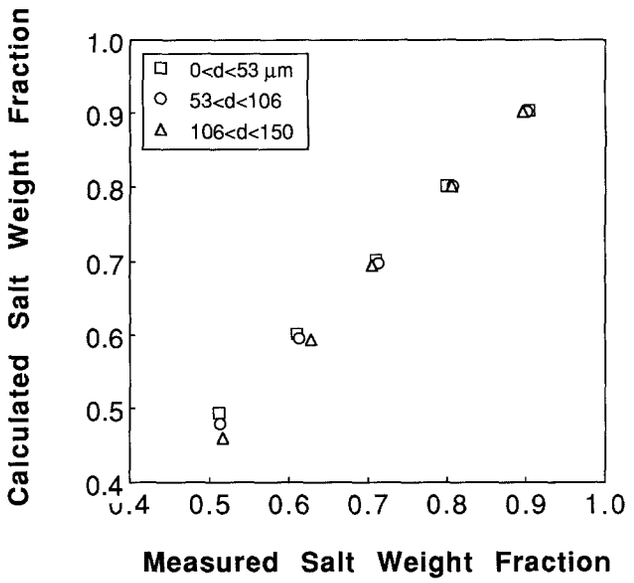

Figure 7 Comparison between the salt weight fraction of PLLA/NaCl composites as measured by t.g.a. and the value calculated from their weight decrease due to salt leaching for foams prepared with different initial salt weight fractions and salt particle sizes

Table 1 Thermal properties of semicrystalline PLLA foams and PLLA/NaCl composite membranes

Type[a]	NaCl (wt%)	NaCl range (μm)	T_m (°C)	ΔH_m (J g^{-1})[b]	X_c
F	70	106–150	181.7	45.8	0.225
C	70	106–150	179.1	54.6	0.268
F	80	106–150	181.0	47.5	0.233
C	80	106–150	179.8	47.3	0.233
F	90	106–150	178.6	47.8	0.235
C	90	106–150	179.3	52.4	0.257
F	90	0–53	177.2	49.9	0.245
C	90	0–53	176.0	44.4	0.218

[a] F = foam; C = composite
[b] Calculated per gram of polymer

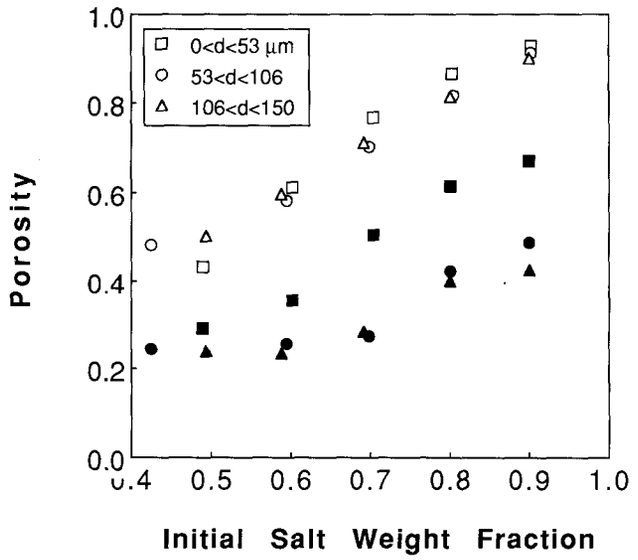

Figure 8 Porosity of semicrystalline PLLA foams (open symbols) and PLLA/NaCl composites (solid symbols) prepared with sodium chloride particles and methylene chloride as a function of the initial salt weight fraction and salt particle size

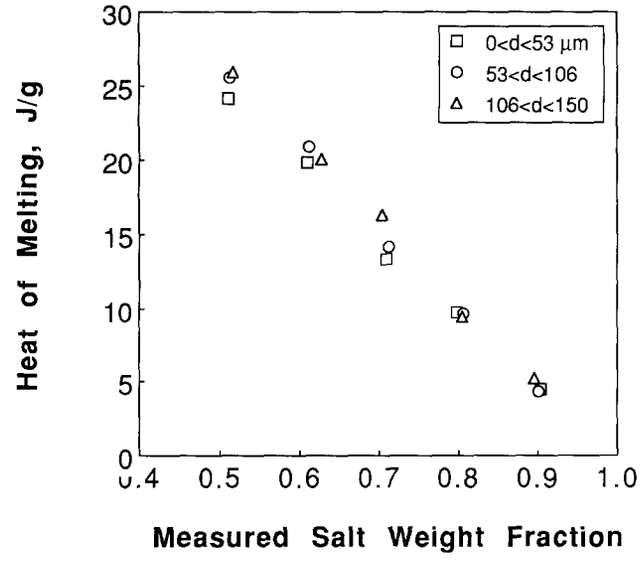

Figure 9 Variation of the heat of melting (per gram of composite) measured by d.s.c. with the salt weight fraction measured by t.g.a. for PLLA/NaCl composites prepared with different initial salt weight fractions and salt particle sizes

the salt weight fraction for all composite samples (*Figure 9*). The same results were also obtained when sodium tartrate or sodium citrate particles were used to prepare PLLA porous membranes (data not shown).

By heat treating PLLA/NaCl composite membranes, we could modify their crystallinity. Using d.s.c., a sample was heated to 200°C and cooled down to 20°C at a constant rate. The heat of crystallization (or the amount of crystallites created) increased as the cooling rate decreased (*Figure 10*). The extent of crystallization was smaller for composite membranes than for salt-free PLLA. Nevertheless, by quenching the composite membranes with cooling rates of 20°C min^{-1} and higher, the polymer became amorphous.

Amorphous PLLA foams were prepared by cooling the PLLA/NaCl composite membranes very quickly from 195 to −195.8°C (temperature of liquid nitrogen). Their d.s.c. thermograms included a cold crystallization (see *Figure 1E*), and equation (7) was used to calculate their degree of crystallinity, which was indeed very close to zero (see *Table 2* for typical values). The glass transition temperature, T_g, did not vary with the processing conditions, and an average of 63.3±3.5°C was obtained. The melting temperature, T_m, was 175.4±1.6°C or 4.1°C lower than that of the semicrystalline foams.

The porosity of the amorphous PLLA membranes increased with the salt weight fraction and was independent of the salt particle size (*Figure 11*). The processing–structure relationship established for the semicrystalline foams was also obeyed by the amorphous foams. Furthermore, the pore morphology of amorphous foams was similar to that of semicrystalline foams. For large salt weight fractions, homogeneous foams with interconnected pores of desired diameter were prepared as demonstrated by SEM in *Figure 12*.

The efficacy of the salt-leaching step was evaluated by n.a.a. The residual sodium weight fraction of semicrystalline and amorphous membranes prepared with sodium chloride decreased as the initial salt weight fraction increased and also as the salt particle size decreased (*Figure 13*). For initial sodium chloride weight fractions of 60 wt% and higher, the residual sodium weight fraction was smaller than 10^{-3} for amorphous membranes and 10^{-4} for semicrystalline ones. Consequently, all PLLA foams prepared by the proposed particulate-leaching technique were essentially salt free. The relatively high sodium content of membranes prepared with 50 wt% salt

Table 2 Thermal properties of amorphous PLLA foams

NaCl (wt%)	NaCl range (μm)	T_g (°C)	T_c (°C)	ΔH_c ($J\,g^{-1}$)	T_m (°C)	ΔH_m ($J\,g^{-1}$)	X_c
70	106–150	63.1	97.9	−39.2	174.1	52.8	−0.004
80	106–150	62.8	99.6	−39.1	173.3	52.0	−0.007
90	106–150	61.0	98.2	−36.8	174.9	53.1	0.014
90	0–53	59.8	94.2	−31.8	174.1	52.5	0.044

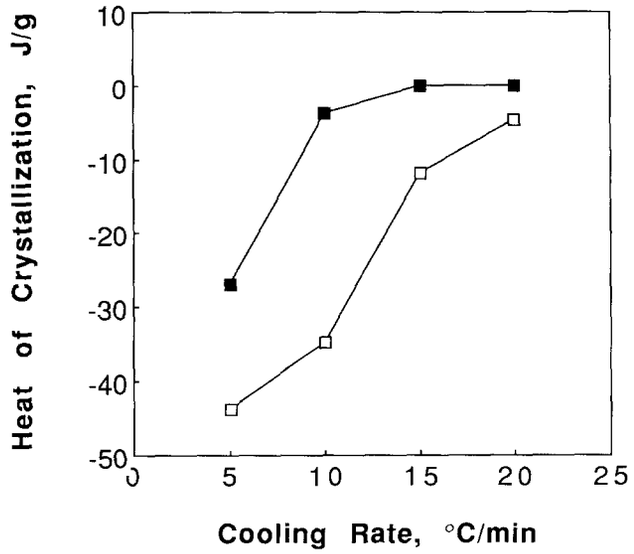

Figure 10 Heat of crystallization (per gram of PLLA) for a PLLA/NaCl composite (■) and a salt-free PLLA foam (□) cooled from 200 to 20°C as a function of the cooling rate. Both samples were prepared with 90 wt% NaCl particles in the size range from 0 to 53 μm

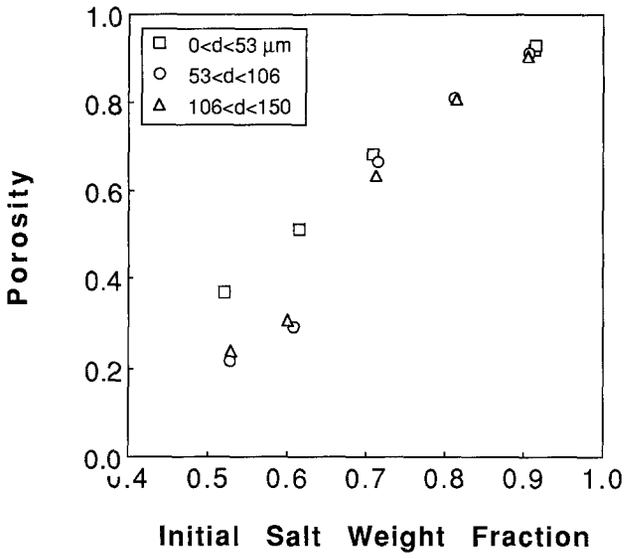

Figure 11 Porosity of amorphous PLLA foams prepared with sodium chloride particles as a function of the initial salt weight fraction and salt particle size

is probably due to the entrapment of salt particles within the dense polymer skin at the membrane surface.

From molecular weight measurements of raw PLLA and semicrystalline foams by g.p.c., we concluded that water did not degrade the polymer while the salt was leached out (*Table 3*). However, the molecular weight of amorphous PLLA foams was smaller than that of raw polymer (*Table 3*). The decrease in the PLLA molecular weight, which also explains the melting temperature decrease, was caused by thermal degradation during heat treatment.

Finally, this new method to process highly porous membranes is not limited to PLLA and can be extended to other polymers and copolymers, such as poly(DL-lactic-*co*-glycolic acid)[23]. It may be particularly attractive for the preparation of biodegradable polymer substrates for cell attachment and transplantation because of its simplicity and flexibility. One can easily tailor the substrate pore size to maximize the vascularization of cell-based artificial organs[29], increase the surface/volume ratio to promote the attachment of anchorage-dependent cells[7,9], or modify the porosity to improve cell seeding and optimize tissue culture[30].

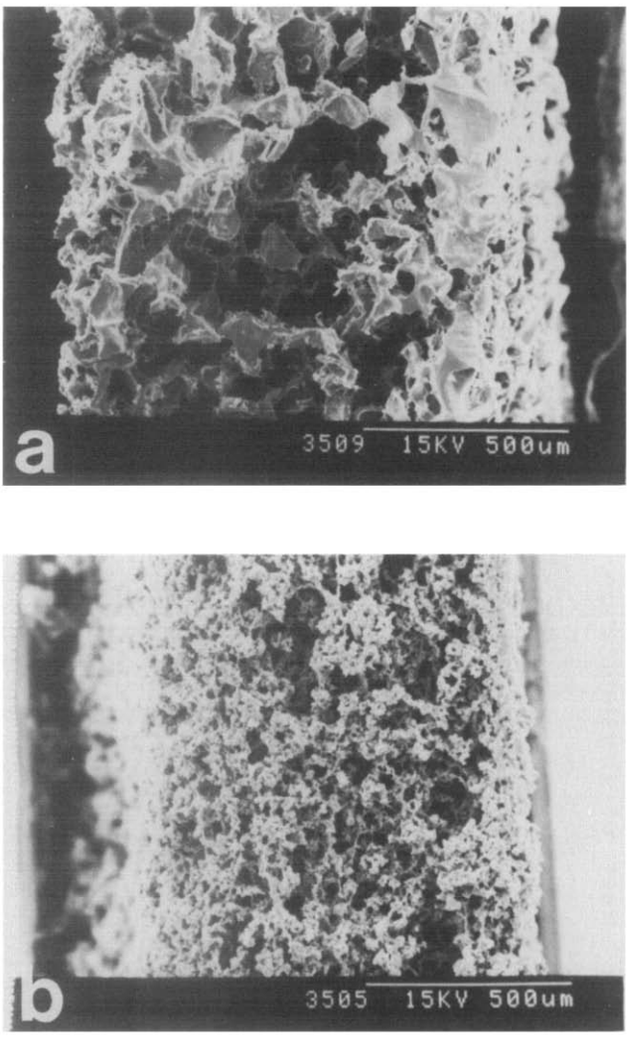

Figure 12 SEM photomicrographs of cross sections of amorphous PLLA foams prepared with 90 wt% NaCl particles in the size range from 106 to 150 μm (a) and from 0 to 53 μm (b)

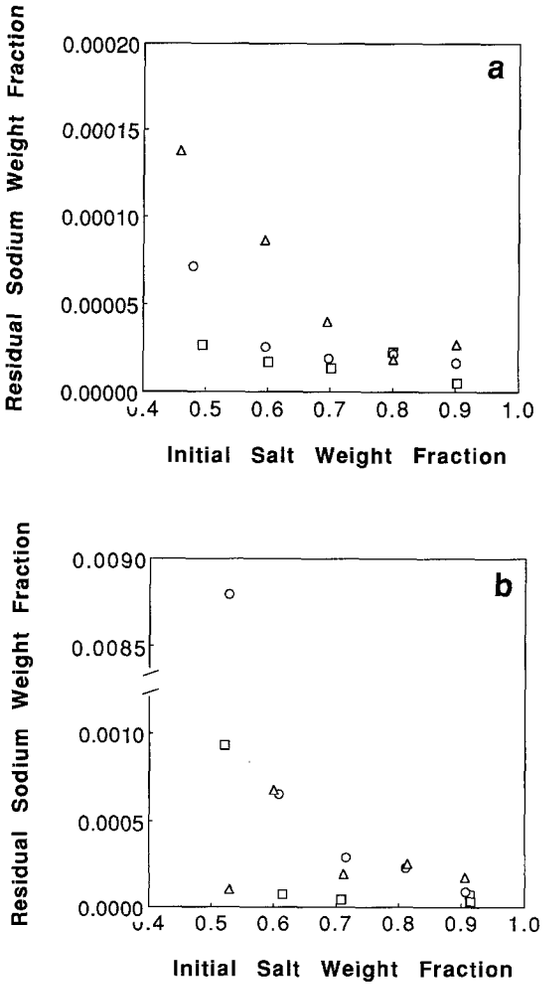

Figure 13 Residual sodium weight fraction (measured by n.a.a.) of semicrystalline (a) and amorphous (b) PLLA foams prepared with sodium chloride particles as a function of the initial salt weight fraction and salt particle size. □, $0 < d < 53\,\mu m$; ○, $53 < d < 106\,\mu m$; △, $106 < d < 150\,\mu m$

Table 3 Number and weight average molecular weights of semicrystalline and amorphous PLLA foams

NaCl (wt%)	NaCl range (μm)	Semicrystalline			Amorphous		
		M_n	M_w	M_w/M_n	M_n	M_w	M_w/M_n
70	106–150	68 100	98 000	1.45	44 600	58 400	1.31
80	106–150	70 300	96 000	1.37	48 600	73 900	1.52
90	106–150	76 300	106 800	1.40	62 200	83 300	1.34
90	0–53	71 700	103 000	1.44	59 600	81 100	1.36
Raw polymer (Polysciences)		75 000	99 600	1.33			

CONCLUSION

A particulate-leaching method was developed to prepare highly porous membranes of poly(L-lactic acid). The utilized materials were non-toxic and were completely removed from the porous membranes. Polymer foams of definite crystallinity, porosity and surface/volume ratio were processed with an open-cell morphology. The foam properties depended only on the initial salt weight fraction and particle size, and were not affected by the salt type or solvent selection.

Membranes of porosity as high as 0.93 with desired surface/volume ratio were produced by leaching salt particles out of porous polymer/salt composite membranes. If the composite membranes had been non-porous, the maximum porosity of the resulting membranes would have been[31] in the range from 0.64 to 0.72. Thus, this method provides a new means for the preparation of highly porous polymers.

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REFERENCES

1 Langer, R. and Vacanti, J. P. *Science* 1993, **260**, 920
2 Vacanti, J. P., Morse, M. A., Saltzman, W. M., Domb, A. J., Perez-Atayde, A. and Langer, R. *J. Pediatr. Surg.* 1988, **23**, 3
3 Ishaug, S. L., Thomson, R. C., Mikos, A. G. and Langer, R. in 'Encyclopedia of Molecular Biology and Biotechnology', VCH Publishers, New York, in press
4 Folkman, J. and Haudenschild, C. *Nature* 1980, **288**, 551
5 Vacanti, C. A., Langer, R., Schloo, B. and Vacanti, J. P. *Plast. Reconstr. Surg.* 1991, **88**, 753
6 Yannas, I. V. in 'Collagen III', CRC Press, Boca Raton, 1988, p. 87
7 Cima, L. G., Ingber, D. E., Vacanti, J. P. and Langer, R. *Biotechnol. Bioeng.* 1991, **38**, 145
8 Colton, C. K. and Avgoustiniatos, E. S. *J. Biomech. Eng.* 1991, **113**, 152
9 Freed, L. E., Marquis, J. C., Nohria, A., Emmanual, K., Mikos, A. G. and Langer, R. *J. Biomed. Mater. Res.* 1993, **27**, 11
10 Puleo, D. A., Holleran, L. A., Doremus, R. H. and Bizios, R. *J. Biomed. Mater. Res.* 1991, **25**, 711
11 Cima, L. G., Vacanti, J. P., Vacanti, C., Ingber, D., Mooney, D. and Langer, R. *J. Biomech. Eng.* 1991, **113**, 143
12 Langer, R., Cima, L. G., Tamada, J. A. and Wintermantel, E. *Biomaterials* 1990, **11**, 738
13 Holland, S. J., Tighe, B. J. and Gould, P. L. *J. Controlled Release* 1986, **4**, 155
14 Singhal, J. P., Singh, H. and Ray, A. R. *Rev. Macromol. Chem. Phys.* 1988, **C28**, 475
15 Langer, R. *Science* 1990, **249**, 1527
16 Wang, H. T., Palmer, H., Linhardt, R. J., Flanagan, D. R. and Schmitt, E. *Biomaterials* 1990, **11**, 679
17 Reed, A. M. and Gilding, D. K. *Polymer* 1981, **22**, 342
18 Greisler, H. *ASAIO Trans.* 1988, **34**, 1051
19 Cooper, M. L., Hansbrough, J. F., Spielvogel, R. L., Cohen, R., Bartel, R. L. and Naughton, G. *Biomaterials* 1991, **12**, 243
20 von Schroeder, H. P., Kwan, M., Amiel, D. and Coutts, R. D. *J. Biomed. Mater. Res.* 1991, **25**, 329
21 Tencer, A. F., Woodward, P. L. and Swenson, J. *NY Acad. Sci.* 1988, **523**, 157
22 Winslow, D. N. in 'Surface and Colloid Science', Plenum Press, New York, 1984, p. 259
23 Mikos, A. G., Sarakinos, G., Leite, S. M., Vacanti, J. P. and Langer, R. *Biomaterials* 1933, **14**, 323
24 Fischer, E. W., Sterzel, H. J. and Wegner, G. *Kolloid Z.Z. Polym.* 1973, **251**, 980
25 Jamshidi, K., Hyon, S.-H. and Ikada, Y. *Polymer* 1988, **29**, 2229
26 Olmez, I. in 'Methods of Air Sampling and Analysis', 3rd Edn, Lewis Publishers, New York, 1989, p. 143
27 Schindler, A. and Harper, D. *J. Polym. Sci., Polym. Chem. Edn* 1979, **17**, 2593
28 Kesting, R. E. 'Synthetic Polymeric Membranes', 2nd Edn, Wiley, New York, 1985
29 Mikos, A. G., Sarakinos, G., Lyman, M. D., Ingber, D. E., Vacanti, J. P. and Langer, R. *Biotechnol. Bioeng.* 1993, **42**, 716
30 Wald, H. L., Sarakinos, G., Lyman, M. D., Mikos, A. G., Vacanti, J. P. and Langer, R. *Biomaterials* 1993, **14**, 270
31 Gibson, L. J. and Ashby, M. F. 'Cellular Solids Structure and Properties', Pergamon Press, New York, 1988